

PROCEDURES

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Determination of Biochemical Oxygen Demand (BOD₅)

5-Day BOD Technique

Reference: Standard Methods, 18th edition, Procedure 5210 B

BOD Analysis: Curses and Cures. 1984. SLOH

Apparatus:

1. 300 ml BOD bottles
2. 2 - 5 liter glass bottle with siphon. *Avoid using detergents to clean these bottles. Periodically clean with bleach water.*
3. $20 \pm 1^\circ\text{C}$ incubator
4. YSI model 54 DO meter
5. Buret

Nutrient Solutions:

1. **Phosphate buffer:** Dissolve 8.5 g KH_2PO_4 , 21.75 g K_2HPO_4 , 33.4 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, and 1.7 g NH_4Cl in approx. 500 ml reagent water. Dilute to 1 L. The pH should be 7.2. Store in 4°C refrigerator. Check before each use for contamination (if there is any indication of biological/microbial growth, discard remaining reagent and prepare fresh).
2. **Magnesium sulfate solution:** Dissolve 22.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in reagent water. Dilute to 1 L.
3. **Calcium chloride solution:** Dissolve 27.5 g CaCl_2 in reagent water. Dilute to 1 L.
4. **Ferric Chloride solution:** Dissolve 0.25 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in reagent water. Dilute to 1 L.

Preparation of Sample:

If any type of chlorination process is employed during treatment, final effluent samples are initially tested for the presence of residual chlorine. Residual chlorine can kill the microorganisms which are critical to measuring BOD.

1. **The diluted sample used to determine BOD must have a pH between 6.5 and 7.5.** For municipal sewage or effluent, the pH range is generally between 5 - 9, but the buffering capacity of the phosphate buffer will often bring the pH of the diluted sample between 6.5 and 7.5. For unknown samples, check the pH of the dilution which uses the most sample to confirm that the dilutions lie in the proper pH range. As needed neutralize samples with 1N sulfuric acid or 1N sodium hydroxide. Do not dilute the sample with the acid or base by more than 0.5% (1.5 ml in a 300 ml BOD bottle).
2. Test final effluent for residual chlorine. Dechlorination is required if a chlorine residual is present when testing is initiated. *See Page BOD - 6.*
3. Samples supersaturated with dissolved oxygen, over about 9.3 mg/l at 20°C , may be encountered during winter months or in localities where algae are actively growing (lagoons). To prevent loss of oxygen during incubation of these samples, the DO should be reduced by shaking the sample or aerating it with filtered compressed air. *These types of samples often have a high concentration of nitrifying organisms, which can lead to bias in BOD results. Nitrification inhibition can be performed, but a permit variance is required if inhibited BODs (CBOD₅) are used for required permit reporting.*
4. Samples of untreated industrial wastes, disinfected wastes (final effluent), high temperature wastes, or wastes with extreme pH values may not contain enough microorganisms to oxidize the biodegradable matter in the samples. Such wastes should be seeded. *See BOD Seeding Procedure on page BOD - 8 and 9.*

Dilution Technique:

1. Estimate the B.O.D. of the sample and select suitable dilutions from the following tables:

Estimated BOD ₅ (mg/L)	Suggested Sample Volumes (mL)
< 5	200, 250, 300
< 10	100, 150, 200
10 - 30	25, 50, 100
30 - 60	15, 25, 50
60 - 90	10, 15, 25

Estimated BOD ₅ (mg/L)	Suggested Sample Volumes (mL)
90 - 150	5, 10, 15
150 - 300	3, 5, 10
300 - 700	1, 3, 5 ***
700 - 1500	0.5, 1, 3 ***
1500 - 2500	0.25, 0.5, 1 ***

Standard Methods provides additional guidance as follows: use less than 3 mL for strong industrial wastes, 3-15 mL for raw and settled wastewater, and 15-75 mL for biologically treated effluent.

When preparing replicate samples for quality control purposes, prepare the replicate at exactly the same dilutions as the original sample. See Helpful Hints #23 (September, 1992) for information regarding the preparation and calculation of BOD replicates.

2. Using a **large-tipped**, volumetric pipette - for samples less than 50 ml - or a graduated cylinder for larger sample volumes, measure the proper amount of well-mixed sample into thoroughly cleaned and rinsed 300 ml bottles.
**** **Dilutions under 3 mL must be made by diluting the waste in a graduated cylinder before pipetting.** ***
3. Dilution water may be prepared immediately before use, or, *except for the addition of the phosphate buffer*, days or weeks ahead of time. Add 1 ml of each nutrient solution per liter of dilution water. The phosphate buffer is the critical nutrient in stimulating contaminating growths so it must be added the day the water is to be used. **Distilled water should be allowed to equilibrate in the incubator or with outside air for at least 24 hours at 20°C before use.** To avoid dust or dirt contamination while allowing oxygenation, use a paper towel, cotton plug, or sponge to cover the bottle opening.

Care is taken to ensure that dilution water is oxygen saturated. The use of aerator stones for this purpose should be discouraged as there is a tendency to develop growths which can prevent dilution water BOD criteria from being met. The best technique is to use compressed air which is passed through glass wool or a filter of some type to prevent the introduction of contaminants.

4. Each BOD bottle is filled by slowly adding sufficient dilution water so that the stopper can be inserted without leaving an air bubble but not so much that there is overflow. The siphon hose must be made of surgical gum (latex rubber), polypropylene or polyethylene to avoid introducing BOD into the dilution water. Tygon and black rubber can add oxygen demand to the water.

When volumes of sample used exceed 150 ml, additional nutrients should be added to the sample bottle. Add an additional 0.1 mL of nutrients for each 50 mL of sample used in excess of 150 mL. For example, if the sample size is 200 ml, an additional 0.2 ml is required.

5. Completely fill two bottles with dilution water to be incubated as blanks.
6. Label each bottle carefully as to sample and volume used. RECORD ON BENCH SHEET.

Incubation and Dissolved Oxygen (DO) Determinations:

1. Calibrate DO meter each day of use and check membrane on probe. Record the barometric pressure each day of analysis. This can be obtained from a barometer in the laboratory. Alternatively, barometric pressure readings can be obtained via the Internet, from a local radio or television news station, or from a local airport. Barometric pressure readings should not be corrected to sea level. **NOTE:** *as a reference, typical barometric pressures range from about 29.6 to 30.3 inches (751.8 mm to 769.6 mm; 1002.36 to 1026.072 millibars) of mercury. Only under severe low pressure systems (seasonal storm systems, e.g., hurricanes or tornadic storms), does barometric pressure drop below 29.0 inches.*
2. Determine the DO of the two dilution water blanks and all sample bottles and record on data sheet as Initial DO.
3. Place the samples and the 2 dilution water blanks in a $20 \pm 1^\circ\text{C}$ incubator for 5 days. Fill water seals with dilution water and cap to reduce evaporation from seals. Check daily, add water to seals if necessary. *Due to the 5 day testing period, certain samples require that set-ups and run-outs of results be performed by different individuals.*
4. Before removing the caps, pour off the water above the cap.
5. After 5 days determine the DO of the two dilution water blanks and the sample bottles.

Calculations:

In general, BOD₅ values less than 2.0 mg DO/L should be reported on DMRs as non-detects (i.e., < LOD). Using the data recorded for un-seeded samples:

$$\text{BOD mg/l} = (\text{Initial DO} - \text{DO}_5) \times \text{Dilution Factor}$$

$$\text{Dilution Factor} = \frac{\text{Bottle Volume (300 ml)}}{\text{Sample Volume}}$$

- Notes:
1. Blank BOD₅. If the DO depletion in the dilution water blanks exceeds 0.2 mg/l, the results of the test are questionable. The results of samples which used the same dilution water the "high" blank should be qualified on the DMR. Do not subtract blank values from sample results.
 2. Dissolved oxygen values of the samples. Only dilutions with DO depletions of at least 2 mg/L, and DO₅ of at least 1 mg/L may be used to calculate the sample's BOD.
 3. Average results from sample dilutions to calculate final sample BOD₅.
 4. See page BOD - 9 for calculations appropriate for seeded samples

If there is no evidence of toxic effects, average the results from all dilutions which meet the minimum oxygen depletion and minimum residual DO requirements. A toxic effect is indicated when BOD increases significantly as the sample dilution increases. This is often referred to as "sliding BODs". Helpful Hints #17 (March, 1991) provides additional guidance for reporting results.

Standardization of DO meter - Winkler Titration Technique

Reference: Standard Methods, 18th edition, Procedure 4500-O C

Reagents:

1. **Manganous sulfate solution:** Dissolve 480 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in reagent water. Filter; dilute to 1 L.
2. **Alkali-iodide-azide reagent:** Dissolve 500 g NaOH and 135 g NaI in reagent water. Dilute to 1 L. Add 10 g NaN_3 dissolved in 40 ml reagent water. *This reagent should not give a color with starch solution when diluted and acidified.*
3. **Concentrated Sulfuric acid**
4. **Standard sodium thiosulfate titrant, 0.0250N:** Purchase commercially.
5. **Starch Solution:** Prepare an emulsion of 5 g soluble starch in a mortar or beaker with a small amount of distilled water. Pour this emulsion into 1 L of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 1.25 g salicylic acid/L and storage at 4°C.

Procedure:

1. Slowly siphon three portions of aerated dilution water into three separate BOD bottles. Avoid adding atmospheric O_2 to dilution water.
2. To two of the three BOD bottles, add 1 ml MnSO_4 solution, followed by 1 ml alkali-iodide-azide reagent. Submerge pipette tips in sample when adding reagents. Rinse tips well between uses.
3. Stopper carefully to exclude air bubbles; mix by inverting bottle several times.
4. When precipitate has settled to about half the bottle volume, carefully remove the stopper and add 1.0 ml conc. sulfuric acid. Re-stopper and mix by gentle inversion until the iodine is uniformly distributed throughout the bottle.
5. Transfer 203 ml of sample into a white 500 ml casserole dish and titrate with 0.0250N sodium thiosulfate to a pale straw color. Add 1-2 ml of starch solution and continue to titrate to first disappearance of the blue color. (200 ml of original dilution water is equal to 203 ml of dilution water plus reagents.)
6. Titrate two of the three samples. Results should be within 0.1 mL if using a buret with increments of 0.05 mL. Calibrate the DO probe with the third bottle.

Standardization of DO meter - Air calibrations

Temperature (°C)	Theoretical Maximum Oxygen Solubility (mg/L)
15	10.084
16	9.870
17	9.665
18	9.467
19	9.276
20 ambient	9.092
21	8.915
22	8.743
23	8.578
24	8.418
25	8.263

Calibration

The Winkler titration is the most accurate method for standardizing a DO meter. If another method is used, it is suggested that the calibration be checked against a Winkler titration occasionally. If a meter is air calibrated, the reading must be corrected for atmospheric pressure. This is best done with a barometer kept in the lab, but another source of this information is a local airport or news station.. Atmospheric pressure readings obtained from an airport are generally corrected for sea level, and must be re-corrected for actual altitude.

If you use a DO meter and probe, it is perhaps easiest if you calibrate according to the manufacturer's instructions. There are two types of oxygen probes available: one of which employs a calibration based on water saturated air, and another based on air-saturated water. The water saturated air procedure involves storing the electrode in a sealed BOD bottle containing a minimal amount of water. For the air-saturated water calibration procedure, you either vigorously shake the solution or bubble air through it.

There are, however, some concepts you should be familiar with to obtain the best quality results.

1. The best way to ensure data quality is to calibrate against a known standard. One way to do this is to use a sample of dilution water which has been saturated with oxygen. By knowing the temperature of the dilution water, and that it is a saturated solution, you then know what your meter should read for calibration. The table above is an abbreviated list of oxygen saturation levels versus temperature. Assuming your dilution water is at 20°C, if you obtain a measured value substantially less than 9.0 mg/L, you will not have an accurate calibration.
2. Some meters also allow the zero calibration standard. By adding an oxygen scavenger (e.g., an excess amount of sodium sulfite) to a sample of dilution water, you can obtain a sample which virtually contains zero oxygen.

Pretreatment of Chlorinated BOD Samples Reagents:

1. **Acetic acid solution, 1+1:** Add 500 ml. of concentrated acetic acid to 500 ml of distilled water.
2. **Potassium Iodide Solution:** Dissolve 10 grams KI in a 100 ml volumetric flask. Bring to volume with distilled water.
3. **Sodium Sulfite Solution, 0.0250N:** Dissolve 1.575 grams anhydrous Na_2SO_3 in a 1,000 ml volumetric flask. Bring to volume with distilled water.

NOTE: This solution is not stable and must be prepared daily.

4. **Starch Indicator Solution (For Analysis with Iodine):** Prepare an emulsion of 5 g soluble starch in a mortar or beaker with a small amount of distilled water. Pour this emulsion into 1 L of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 1.25 g salicylic acid/L and storage at 4°C.

Procedure:

1. Conduct a chlorine residual analysis on a portion of the sample collected. *Potassium iodide/starch paper can be used as a quick qualitative test for residual chlorine.* If no residual is found, proceed with the BOD analysis utilizing seeded dilution water. If a residual is found, proceed with the following steps before initiating the BOD test.
2. Determination of Appropriate Volume of Sodium Sulfite
 - a. Obtain a 200 ml portion of the sample to be tested.
 - b. Add 10 ml of 1+1 acetic acid solution
 - c. Add 10 ml of potassium iodine solution
 - d. Add 2 ml starch
 - e. Titrate with 0.0250N sodium sulfite. The end point has been reached when a clear color persists after complete mixing.
 - f. Measure volume of 0.0250N sodium sulfite used.
3. Sample Pretreatment
 - a. Obtain another 200 ml portion of the same sample used in Step 2.
 - b. Add to the sample the same volume of 0.0250N sodium sulfite solution that was determined in Step 2.e and mix.
 - c. Retest for residual chlorine after allowing the sample to stand for 10-20 minutes.
 - d. If no residual chlorine is present, proceed with the BOD analysis. Samples which have been chlorinated must be seeded.

Preparation of Glucose - Glutamic Acid Standard (GGA)

Reagents:

Note: glucose/glutamic acid solution can be purchased commercially, but needs to be prepared such that the GGA concentrations are equal to 150 mg/L each.

1. Reagent grade glucose
2. Reagent grade glutamic acid

Procedure:

1. Dry reagent grade glucose and glutamic acid at 103°C for 1 hour and cool for one hour in the desiccator.
2. Dissolve 150 mg (0.150 g) of glucose and 150 mg (0.150 g) of glutamic acid in distilled water and bring up to 1 L.

Note: This solution will become contaminated quickly and must be used immediately unless the following is done. Place into each of several milk dilution bottles or capped test tubes the quantity of the GGA standard which is used in one day. Seal the bottles and sterilize them. These sterilized portions can then be cooled and stored at 4°C. When a known standard is run, 6 ml of GGA standard from one of the sealed/sterilized containers is added to each BOD₅ bottle and the bottles are filled 3/4 full with dilution water. (This is critical! 198 ± 30.5 mg Oxygen/L is based on a 2 % dilution of GGA (6 mL/300). It is important not to use multiple dilutions which use other than 6 mL). Seed is then added and the bottle is filled with dilution water. These bottles are incubated and BOD is determined similar to sample bottles.

3. The acceptable BOD₅ value of the standard is 198 ± 30.5 mg/l. If the calculated result falls outside this range the cause of the problem must be identified. Sample results obtained using the same seed or dilution water as the standard must be qualified. Once the problem is corrected another known should be set up immediately.

BOD Seeding Procedure

Preparation of Seed:

1. Collect a raw influent grab sample the day before performing the test. *If the influent contains significant industrial loading, settled mixed liquor may provide a better seed than raw influent. If used for seed, settled mixed liquor does not need to be incubated at 20°C overnight. Seed can also be commercially obtained. There are at least two products widely in use: BioSeed™, and PolySeed™.*
2. Place sample in incubator (20°C) overnight.

Preparation of Seed Controls:

Table 8 gives general directions for determining the amount of seed to add to seed controls and samples.

1. Take the incubated raw influent sample out of the incubator -- DO NOT MIX.
2. Pipet 3, 5, and 7 ml of the clear supernatant into three BOD bottles respectively. Use other volumes of supernatant based on the strength of your system. You MUST use at least two different dilutions.
3. Fill these three bottles with BOD dilution water.
4. Determine the initial dissolved oxygen on each of the three bottles.

Preparation of Seeded BOD Samples:

1. Fill the bottles approximately 1/3 - 1/2 with dilution water.
2. Pipet 2 ml of the supernatant into each of the BOD sample bottles that will require seeding.
3. Add the appropriate amount of sample to each of the bottles.
4. Complete the filling of the BOD bottles with dilution water.
5. Determine the initial dissolved oxygen (IDO) on each of the bottles.

Calculation of Seed Correction:

1. Determine the 5 day DO concentration on each of the seed controls.
2. Use the same rule for DO depletion as in all other BODs (at least 2.0 mg/L DO depletion and at least 1.0 mg/L residual DO (after 5 days) (*Standard Methods, 18th edition*)).
3. If none of the bottles attain a proper depletion, adjust the amount of seed addition accordingly in subsequent tests.
4. For each seed control dilution, the mg DO used per ml seed =
$$\frac{(\text{IDO} - \text{DO}_5 \text{ for seed control})}{\text{ml seed in seed control}}$$
5. If two seed controls meet the DO depletion criteria, calculate the average mg DO depleted/mL seed.
6. Seed correction =
$$(\text{mg DO/ ml seed in seed control}) \times (\text{ml seed added to samples}^@)$$

@ If the seed is diluted before it is added to the sample bottles, the ml of the diluted seed added to the sample bottle must be multiplied by a dilution factor. (Ex. If 10 ml seed + 90 ml water are mixed to produce the seeding material, the dilution factor is 1/10.)

7. If the seed correction does not fall in the range of 0.6-1.0, but the seed controls met the DO depletion criteria, the amount of seed used in the sample bottles will have to be adjusted in subsequent tests.

Calculation of BOD in sample:

$$\text{BOD}_5 = \text{BOD mg/l} = [(\text{IDO} - \text{DO}_5) - \text{seed correction}] \times \text{dilution factor}$$

$$\text{* dilution factor} = \frac{300}{\text{sample size (mL)}}$$

Table 8 - BOD SEED DILUTION GUIDELINES

(1)	(2)	(3)	(4)
Estimated BOD of seed	Dilutions for Seed Control	# mL seed per BOD bottle	# mL diluted seed (10 mL seed + 90 mL water)
30	15, 25, 50	6 - 10	NA
50	15, 25, 50	4 - 6	NA
100	5, 10, 15	2 - 3	NA
150	5, 10, 15	1 - 2	NA
250	3, 5, 10	1	6 - 10
500	1, 3, 5	0.5	5

If the BOD of the seed is 150 mg/L or less, the seed may be added directly to the BOD samples without dilution. If dilution is necessary, use volumes noted in column (4). Set up the seed control dilutions as shown in column (2). Prepare seed controls with seed at full strength.

Seed Correction Sample Calculation:

SEED CONTROL BOTTLE	IDO	DO ₅	DEPLETION	# mL SEED IN BOTTLE	mg DO/mL SEED
A	8.5	0.3	8.2	30	---
B	8.4	1.6	6.8	20	0.34
C	8.4	4.3	4.1	10	0.41

NOTE: Bottle A is not used due to the insufficient final DO. There must be a residual DO of at least 1.0 mg/L after 5 days.

$$(0.34 + 0.41)/2 = 0.375 \text{ mg DO/ml seed}$$

If 2 ml undiluted seed added to each sample bottle,

$$\text{seed correction} = (0.375 \text{ mg DO/ml seed})(2 \text{ ml seed}) = 0.75 \text{ mg DO}$$

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Determination of Total Suspended Solids

Gravimetric Technique, Dried at 103 - 105 °C

Reference: Standard Methods, 18th edition, Procedure 2540 D

Apparatus:

1. Glass fiber filter discs, without organic binder, Whatman GF/A
2. Aluminum weigh dishes
3. Membrane filter funnel and suction flask
4. Drying oven: 103 - 105°C
5. Desiccator: Indicating Drierite™ should be blue. Regenerate in 204°C oven at least one hour if pinkish color observed. *If a conventional desiccator is used, it should be checked regularly to ensure that a proper seal is obtained. Apply stopcock grease as necessary to obtain improve sealing ability.*
6. Vacuum pump

Procedure:

1. Preparation of Filters

Filters are pre-washed, dried, and weighed with the previous day's sample run and stored in a desiccator.

 - a. Prepare a membrane-filter-type assembly (47 mm filters) for use. *Gooch crucibles (bitumen type) or a Buchner funnel may be used.*
 - b. Filters should not be touched with fingers but rather should be handled with forceps or rubber gloves. Filters should be placed in funnels with their rough sides up. Filters should not be set directly on drying racks to avoid sticking. Mark aluminum weigh dishes used to support filters with an identifying number or symbol. Some labs have developed unique filter supports which allow for better convection during drying. *The most important consideration is to ensure that during transport (e.g., from desiccator to balance) residue material is not "shaken loose" from the filter.*
 - c. Rinse under suction with at least 3 - 20 ml portions of reagent water. *This is an important step! This procedure is necessary to dislodge and dissolve any extraneous particulates on the filter.*
 - d. Place filter plus aluminum weigh boat into oven and dry at 104 ± 1 °C for at least 1 hour.
 - e. Remove from oven and place in desiccator to cool for 20-30 minutes. Weights should be taken immediately after cooling to obtain the "driest" weight. *This is especially true during humid months, when the dried residue can quickly absorb moisture (and thus weight) from the air.*
 - g. Zero the balance.
 - h. Remove filter from desiccator and weigh. RECORD WEIGHT. Filter may then be returned to the desiccator until needed. *The cycle of drying, cooling, desiccating, and weighing (STEPS 1d. - 1g.) should be repeated until a constant weight is achieved or the loss between two successive readings is less than 0.0005 g.*
2. Sample Preparation
 - a. Place tared filter on membrane filter apparatus, wet down filter disc with about 10 ml distilled water to seat it.
 - b. Apply vacuum at approximately 20 psi and pipette suitable volume of sample into crucible. (On clear samples use up to 2 L of sample. On turbid samples use smaller volumes. Typical amounts to use are 500 ml for clear final effluent, 25 or 50 ml on raw sewage and primary effluent, and 5 to 25 ml on mixed liquor.) *It is suggested that sample volumes over 50 ml be measured with a graduated cylinder.*
 - c. Rinse the inside of the pipet or graduated cylinder with a squirt bottle of distilled water. Add this to the filter.

- d. **RECORD VOLUME OF SAMPLE FILTERED.** The volume filtered should be sufficient to give a solids weight of at least 0.001 g (1 milligram). Because excessive solids on the filter may form a water-entrapping crust, limit the sample size to that yielding no more than 0.2 g (200 mg)TSS.
- e. Place in oven and dry at 104 ± 1 °C at least 1 hour, place in desiccator to cool for 20 - 30 minutes.

NOTE: *The sample residue should be evenly distributed over the entire filter surface. The presence of “blank spots” on the filter, where no residue exists, indicates blockages in the micro screen support on which the filter pad rests during filtration.*

- f. Remove the sample from the desiccator and weigh. **RECORD WEIGHT.** Subtract the weight of the filter obtained in step 1.e above from this weight. Every three months, redry and reweigh a sample to verify that it is dried to constant weight. This is done by repeating the cycle of heating, drying and cooling in the desiccator, and weighing until a constant weight is achieved. The value of the weight should change by no more than 4 % or 0.5 mg, whichever is less. The verification that you are drying to a constant weight every three months may not be necessary if the sample is dried overnight. However, you should occasionally verify that drying overnight meets the constant weight definition as described above. *The method specifies that all samples be re-dried and re-weighed to confirm that they have reached constant weight. It is not necessary to check that every sample has reached constant weight, as long as the analyst periodically confirms this fact.*
- g. Calculate suspended solids (mg/l) using the following formula:

Suspended Solids (mg/l or ppm) =

$$\frac{[\text{Wt. of Solids (g) from step 2.f}] \times [1,000,000]}{\text{Volume of Sample filtered (ml)}}$$

It is a good idea to set up your benchsheet to include a column for the amount of residue (final weight minus tare weight). This column can then be quickly scanned to verify that each sample is associated with at least 1 mg of residue, but not more than 200 mg of residue.

If less than 1 mg of residue is obtained, you will need to filter a larger volume of sample. However, unless your permit limit is less than 5 mg/L, there is little need to filter more than 1 L of sample. If more than 200 mg of residue is obtained, the sample should be re-analyzed using a smaller sample volume.

Determination of Ammonia

Ion Selective Electrode (ISE) technique

References: Standard Methods, 18th edition, Procedure 4500-NH₃ F
Manufacturer's manual

Chlorine is allowed to dissipate but a residual test is performed to ensure that chlorine is not present at the time of analysis. Neither solids concentrator nor final effluent samples are distilled prior to analysis. Comparison of distilled and non-distilled samples showed that distillation did not significantly affect the results for concentrator decant samples. Raw effluent is not distilled based on the State Laboratory of Hygiene's study, "Evaluation of Preliminary Distillation Prior to Ion Selective Electrode Determination of Ammonia in Municipal Wastewater Effluent".

Apparatus:

1. Orion model 701A Digital pH/mV meter or equivalent
2. Orion Model 95-12 ion selective electrode or equivalent
3. Magnetic stirrer

Reagents:

1. **Ammonia free water:** All reagents and dilutions must be made with ammonia free water. Ammonia free water is prepared by passing distilled water through an anion/cation exchange column immediately before use.
2. **Sodium Hydroxide, 10N:** Dissolve 400 g NaOH in 800 ml reagent water. Cool and dilute to 1 L.
3. **Stock ammonium chloride solution, 1000 ppm NH₃-N/L:** Dissolve 3.819 g anhydrous NH₄Cl, dried at 100°C, in water. Dilute to 1 L.

NOTE: For many laboratories, it is easier to purchase commercially-prepared ammonium chloride standards solutions rather than preparing these standards in the lab. It is recommended that ammonium chloride be purchased from two different suppliers, each of which is then used to prepare a 1000 mg/L stock standard. One of these stocks is used to prepare calibration standards, while the other is used as a spiking solution to prepare spikes. If the same solution that is used to prepare calibration standards is also used to prepare spiked samples, errors made in the preparation of the stock standard cannot be easily identified.

4. **Dilute ammonium chloride solution, 100 ppm NH₃-N/L:** Dilute 10 mL of the stock ammonium chloride solution to 100 mL with deionized water.

NOTE: This solution is used to prepare spiked samples. Refer to the previous footnote for more information.

Distillation Procedure:

NR 219, Wis. Adm. Code, requires laboratories either to distill all samples prior to analysis for ammonia or prove that distillation is not needed. The Wisconsin State Laboratory of Hygiene in cooperation with the Wisconsin DNR conducted a study which showed that municipal wastewater effluent tested for ammonia by ion selective electrode did not require prior distillation. While it is unlikely that domestic municipal effluent samples will require digestion, it is likely that municipal plants that receive industrial or pre-treatment wastes will require an initial distillation. Paper mills also have a unique analytical matrix that typically requires a distillation to obtain good quality results. Essentially, if your treatment plant deals with anything other than domestic municipal wastewater effluent, the variance granted to the State Lab of Hygiene waiving the distillation requirement does not apply. Other facilities are required to submit similar documentation using their own wastewater to substantiate waiving the distillation requirement.

Municipal wastewater treatment labs may use the State Laboratory of Hygiene's study instead of conducting their own studies to prove that distillation is unnecessary before ammonia analysis by ion selective electrode. Copies of the State Laboratory of Hygiene's study, "Evaluation of Preliminary Distillation Prior to Ion Selective Electrode Determination of Ammonia in Municipal Wastewater Effluent", can be obtained from the Wisconsin DNR's, Bureau of Integrated Science Services, Box 7921, Madison, Wisconsin 53707-7921.

The detailed procedure for distillation is included as an appendix to this document. The distillation is performed at a pH of 9.5 to prevent hydrolysis of some cyanates and organic nitrogen compounds. A 500 mL sample aliquot is distilled. A borate buffer solution is added and the sample pH adjusted to pH 9.5 with 1N NaOH. Distillation proceeds at a rate of about 6 to 10 mL per minute until at least 200 mL of distillate are collected. The choice of

determinative method (i.e., ion selective electrode, titrimetry, or Nesslerization) dictates what specific receiving solution is contained in the receiving flask.

Analysis Procedure:

1. Check Electrode Slope
 - a. Put 100 ml reagent water and 1 ml 10M NaOH solution into a 150 ml beaker. Turn function switch of 701A to REL MV position. Place electrode in the solution. Use magnetic stirring throughout the procedure.
 - b. Pipet 1 ml 1000 ppm standard into the solution. Set the reading to 000.0 by adjusting the calibration control.
 - c. Add 10 ml 1000 ppm standard to the solution. Correct electrode operation is indicated by a reading of $(-57 \pm 3 \text{ mV})$ (i.e., -54 to -60 mV), assuming the solution temperature is between 10 and 25°C. If the change in potential is not within this range, check the Troubleshooting section of the electrode and specific ion meter manuals. **Do not continue with the analysis until the proper change in potential is obtained.**
2. Preparation of Calibration Standards
 - a. Prepare a standard curve each day samples are tested.
 - b. Prepare standards at 20, 2, and 0.2 mg NH₃-N per liter. *This calibration range will be appropriate if your sample concentrations do not exceed 20 mg/L. If your samples exceed this level, additional standards (e.g., 50 ppm) may be required to extend the calibration without the need for sample dilution.*
 - ♦ To prepare the **20.0 ppm** standard: pipette 2.0 ml of the 1000 ppm stock solution into a 100 ml volumetric flask and bring to volume with reagent water.
 - ♦ To prepare the **2.0 ppm** standard, pipette 2.0 ml of the 100 ppm standard into a 100 ml volumetric flask and bring to volume with reagent water.
 - ♦ To prepare the **0.2 ppm** standard, pipette 10.0 ml of the 2.0 ppm standard into a 100 ml volumetric flask and bring to volume with reagent water.
 - c. The standards (and samples) should be at room temperature before the test is run. A 1°C difference in temperature will give rise to about a 2 % measurement error.

3. Calibration

Prior to 1993, calibration could be performed using only one or two standards. Unfortunately, the reference method that allowed this option has been removed from the list of approved wastewater methods at the federal level. Consequently, the method has also been deleted from chapter NR 219, Wis. Adm. Code. Currently, the laboratory certification and registration program specifies:

NR 149.14 Quality Control. (3)(b)A calibration shall consist of at least 3 standards and a blank except as allowed in approved methods using ion selective electrodes or inductively coupled plasma.

All currently approved ion selective electrode methods require that calibration curves for ammonia be constructed using at least 3 standards and a blank. Many laboratories have indicated that they are using Standard Methods method 4500-NH₃ D, which actually requires the use of five standards. If your ion meter is not capable of using at least three standards in the calibration process, then you can construct a graph similar to that used for phosphorus, but using semi-logarithmic graph paper.

The preferred alternative is to calculate a linear regression. This will result in more accurate and trace-able results. Since ion selective electrodes require a logarithmic conversion, a linear regression of the log of concentration versus millivolt response is necessary. Contact your certification officer if you need assistance performing this calculation.

Refer to the individual procedures for preparing calibrations (pages NH₃-5 to NH₃-9)

- a. For all calibration procedures, place the electrode in the standard solution. Add 1 ml of 10 M NaOH for each 100 ml of standard. Turn on the magnetic stirrer. Keep the stirrer at the same speed for both standards and samples. Place a piece of cardboard between the stirrer and the beaker to prevent heating of the solutions. ***Do not add NaOH before immersing electrode because ammonia may be lost from the basic solution!***

NOTE: Below 1 ppm $\text{NH}_3\text{-N}$, the relationship between concentration and potential (mV) is not linear. To accurately measure ammonia levels in the 0.1 to 1.0 ppm range, at least two additional standards should be prepared and plotted, 0.1 ppm (10 ml of 1.0 ppm standard diluted to 100 ml) and 0.5 ppm (5 ml of 10 ppm standard diluted to 100 ml).

- b. Re-calibrate every two hours. If ambient temperature has changed or a check standard is off by 10% or more, repeat the entire calibration procedure.

4. Sample Measurement

- a. Rinse the electrode and place it in 100 ml of sample. Add 1ml of 10N NaOH is added and begin stirring. Record the millivolt response from the meter and determine the concentration using the calibration curve. Samples with high levels of dissolved species ($> 1 \text{ M}$) may give inaccurate results (refer to specific ion meter/electrode manuals). *Wait at least 5 minutes to record millivolts for samples and standards containing less than 1 mg/L $\text{NH}_3\text{-N}$.*

5. Sample Quantitation

a. **Direct Instrument readout**

If your ion meter is capable of using three or more calibration standards, then this is perhaps the easiest way to generate data.

b. **Hand-drawn curves (semilogarithmic and relative mV techniques)**

The best way to document this process is to make draw ruler lines from the absorbance (vertical) axis across to the point where it meets your hand drawn calibration line. Then drawn another ruler line down from this point until it meets the concentration (horizontal) axis.

c. **Linear Regression or Computer Spreadsheet Applications**

Multiply millivolt response by the slope and add the intercept. Convert this value by taking the antilog of it; alternatively, use a calculator or computer spreadsheet to obtain the value equivalent to raising 10 to the power of the linear regression value. *Refer to the section on Linear Regression (page $\text{NH}_3\text{-6}$).*

6. Other Analytical Methods

a. **Standard Methods 4500-NH3 E (Titrimetric Method)**

Use only on distilled samples. Titrate ammonia in distillate w/ 0.02N H_2SO_4 until the indicator (in the boric acid) turns a pale lavender. Calculate the mg/L as $\text{NH}_3\text{-N}$ by subtracting the amount of titrant required for a blank from that required for a given sample. Multiply the blank corrected titrant volume by 280, and divide the final result by the volume of sample used (in mL). This works because 1.00 mL of 0.02N H_2SO_4 is equivalent to 280 ug of Nitrogen as N.

Mixed indicator solution and the indicating boric acid should be prepared fresh monthly. If not purchased commercially, the 0.02N H_2SO_4 should be standardized (using primary standard grade sodium carbonate, Na_2CO_3 , as per the alkalinity procedure).

b. **Standard Methods 4500-NH3 C (Nesslerization)**

Not recommended, and the EPA is expected to eliminate this procedure due to reagent toxicity. The Nessler reagent contains 100 g mercuric iodide per liter. The method requires a 2 mL addition of the Nessler reagent to 50 mL sample in Nessler tube, which amounts to 88 mg Hg in 50 mL (1760 mg/L as Hg). Alternatively, the laboratory can neutralize boric acid with NaOH and only use 1 mL Nessler reagent. Record absorbance at 425 nm.

Direct Calibration (manual) using Semi-logarithmic graph paper

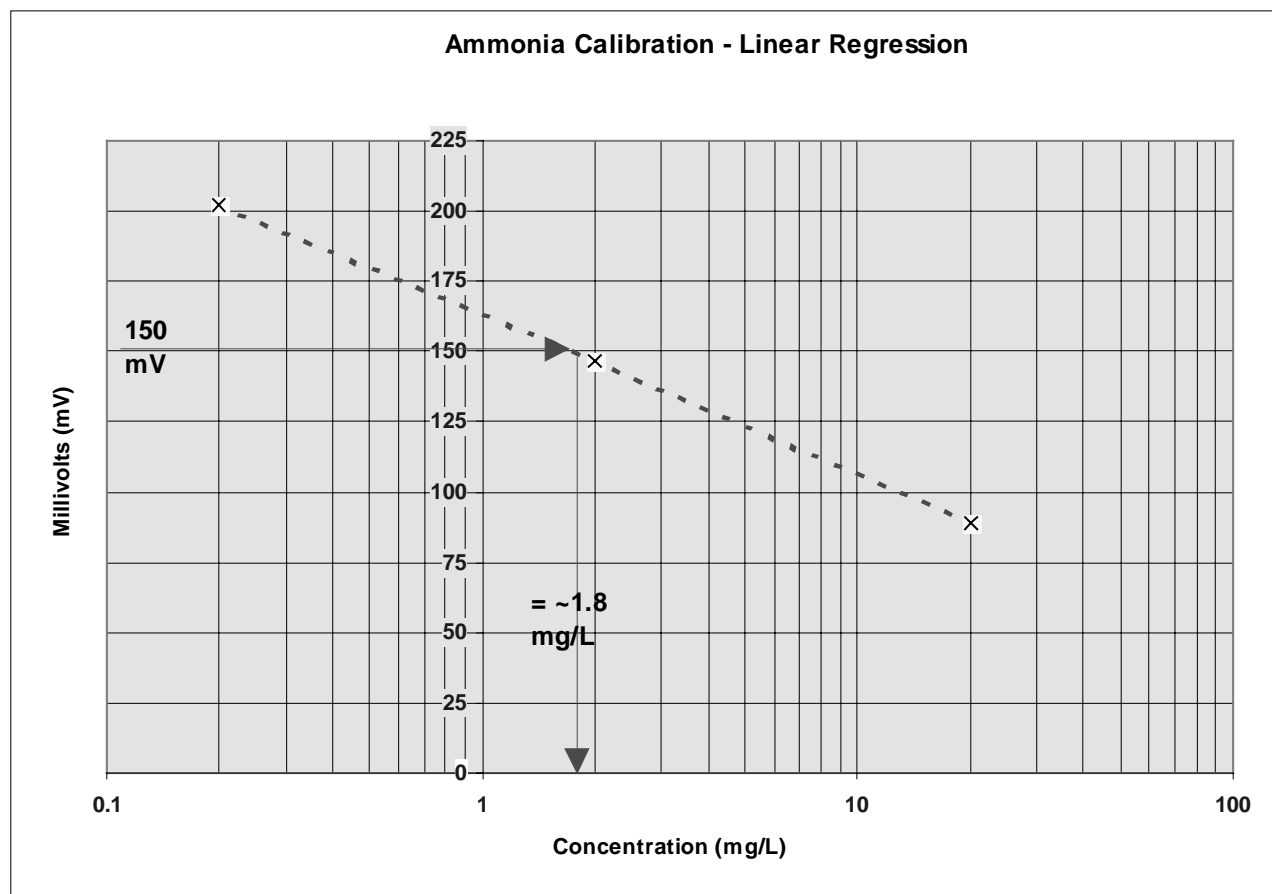
<u>mg/L</u>	<u>mV</u>
0.2	202
2	146.7
20	89.1

Plot, by hand, the millivolt (mV) reading (vertical axis) against concentration (horizontal axis) on semi-logarithmic (semi-log) graph paper. Semi-log paper is available with a different number of “cycles”. Each cycle represents an order of magnitude change, or a power of ten (10).

Thus, if you are plotting the calibration data above, you will need to go from 0.1 -- the next lower power of 10 below the low calibration standard—up to 100 --the next higher power of 10 above the highest calibration standard. This represents 3 cycles (0.1 to 1.0, 1.0 to 10, and 10 to 100).

The best way to document this process is to make draw ruler lines from the absorbance (vertical) axis across to the point where it meets your hand drawn calibration line. Then drawn another ruler line down from this point until it meets the concentration (horizontal) axis. In the example below, a sample response of 150 mV translates to a concentration of about 1.8 mg/L.

To obtain better accuracy, using a millimeter ruler, one can extrapolate the distance from the 0.1 to the line drawn down relative to the distance (in mm) from the 0.1 to the 0.2 line. In the example below, a concentration of 1.8 is obtained if the user measures the distance from the “1” to the arrow to be 9 mm, and the distance from the “1” to the line associated with 2 (mg/L) as 10 mm. $(9/10) * 2 = 1.8$ mg/L.

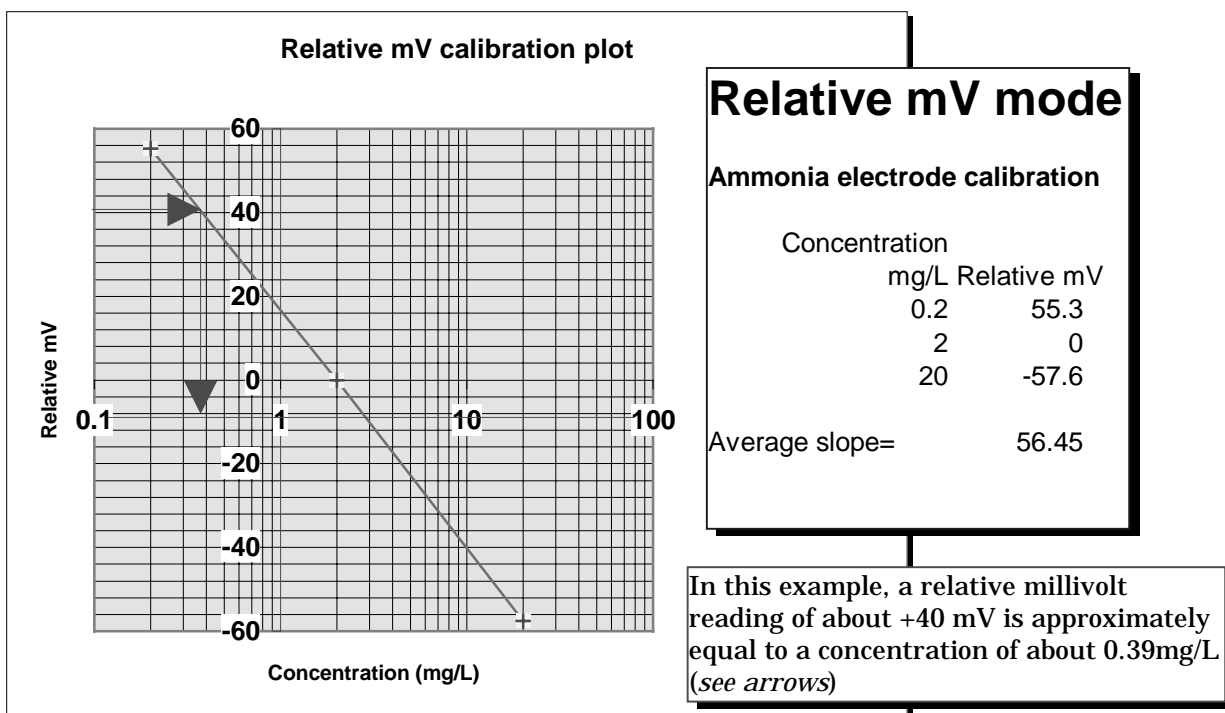


Ammonia Calibration using Relative Millvolts

- a. First, the electrode is placed in the midpoint (2.0 ppm) standard. Add 1 ml of 10 M NaOH for each 100 ml of standard. *The pH at this point should be above 11.* Turn on the magnetic stirrer. Keep the stirrer at the same speed for both standards and samples. Place a piece of cardboard between the stirrer and the beaker to prevent heating of the solutions. **Do not add NaOH before immersing electrode because ammonia may be lost from the basic solution.** The function switch should be set to REL MV. Set the reading to 000.0 with the calibration control.
- b. Rinse electrode and place it in the low calibration (0.2 ppm) standard, which should be prepared at a concentration near the LOQ (i.e., $3.33 \times \text{LOD}$). Add 1 ml of 10N NaOH for each 100 ml of standard and begin stirring. *At such low concentrations, the meter may take up to 15 minutes to stabilize.* Record the millivolt reading (*it should be a negative value*).

NOTE: Below 1 ppm $\text{NH}_3\text{-N}$, the relationship between concentration and potential (mV) is not linear. To accurately measure ammonia levels in the 0.1 to 1.0 ppm range, at least two additional standards should be prepared and plotted, 0.1 ppm (10 ml of 1.0 ppm standard diluted to 100 ml) and 0.5 ppm (5 ml of 10 ppm standard diluted to 100 ml).

- c. Rinse the electrode place it in the uppermost (20.0 ppm) calibration standard. Add 1 ml of 10N NaOH for each 100 ml of standard and begin stirring. Record the millivolt reading.
- d. *Some meters that are limited to using only two calibrations standards in direct readout mode, can be tricked into using three standards by using the relative millivolt technique.* Otherwise, plot, by hand, the millivolt (mV) reading (vertical axis) against concentration (horizontal axis) on semi-logarithmic graph paper. The slope of each of the two segments (equal to the absolute value of the mV response obtained for the low and the high standards) should both be 57 to 60.



Calibration using Linear Regression

A linear regression has the formula: $Y = mX + b$

where Y = Dependent variable, Instrument response

X = Independent variable, concentration

m = the slope

b = the intercept

<u>mg/L</u>	<u>Log of mg/L</u>	<u>mV</u>
0.2	-0.698970	202
2	0.301030	146.7
20	1.301030	89.1

The formula to calculate the **slope** manually is:

$$\text{slope} = \frac{N \sum (X_i Y_i) - \sum X_i \sum Y_i}{N \sum X_i^2 - (\sum X_i)^2}$$

where: N = the number of standards (3)

$\sum (X_i Y_i)$ = (e.g., $[-0.69897 \times 202] + [0.301 \times 146.7]$,.....)

$\sum X_i$ = (e.g., $[-0.69897 + 0.301]$,....)

$\sum Y_i$ = (e.g., $[202 + 146.7]$,....)

$\sum X_i^2$ = (e.g., $[0.4886 + 0.0906]$,....)

$(\sum X_i)^2$ = (e.g., $[-0.69897 + 0.301]^2$)

$$\begin{aligned}
 \therefore \text{slope} &= \frac{\begin{array}{ccccc} 3 & X & 18.8909 & \text{---} & 0.90309 \\ 3 & X & 2.2719 & \text{---} & 0.90309 \end{array}}{\begin{array}{ccccc} 56.6728 & \text{---} & 395.3728 \\ 6.815572 & \text{---} & 0.815572 \end{array}} \\
 &= \frac{-338.7}{6} \\
 &= \mathbf{-56.45}
 \end{aligned}$$

The formula to calculate the **intercept** manually is:

$$\begin{aligned}
 \text{Intercept} &= \frac{(\sum Y_i - \text{slope} \times \sum X_i)}{N} \\
 &= \frac{\begin{array}{ccccc} 437.8000 & \text{---} & -56.45 & X & 0.903090 \\ & & 3 & & \end{array}}{3} \\
 &= \frac{\begin{array}{ccccc} 437.8000 & \text{---} & -50.9794 & & \end{array}}{3} \\
 &= \frac{488.7794}{3} \\
 &= \mathbf{162.9265}
 \end{aligned}$$

The formula to calculate the **correlation coefficient (r)** manually is:

$$\begin{aligned} \text{Correlation coefficient} &= \frac{\sum [(X_i - \bar{X})(Y_i - \bar{Y})]}{\sqrt{\sum [(X_i - \bar{X})^2] * \sum [(Y_i - \bar{Y})^2]}} \\ &= \frac{-112.9000}{\sqrt{2.000000 * 6374.087}} \\ &= \frac{-112.9000}{112.90780} \\ &= -0.999931 \end{aligned}$$

For a calibration to be acceptable, the correlation coefficient must be at least 0.995 (*ignoring the sign*)

Given the following sample data:

<u>mg/L</u>	<u>mV</u>	<u>Regression Line</u>	<u>Log of mg/L</u>	<u>mV</u>
0.2	202	204.8862	-0.698970	202
2	146.7	149.5862	0.301030	146.7
20	89.1	91.98621	1.301030	89.1

$r = -0.9999$
 slope = -56.45 *** this is the electrode slope
 intercept = 162.926

Test unknown mV: 205.6 → **antilog** = -0.75595258

<u>Sample #</u>	<u>mV</u>	<u>log conc.</u>	<u>Concentration mg/L</u>
Final effluent	205.6	-0.75595	0.175407

Step 1. Subtract the intercept from the sample mV: $205.6 - 162.926 = 42.674$

Step 2. Divide the result of Step 1 by the slope: $42.673 \div (-56.45) = -0.75595$

This gives you the ANTIlog of the sample concentration

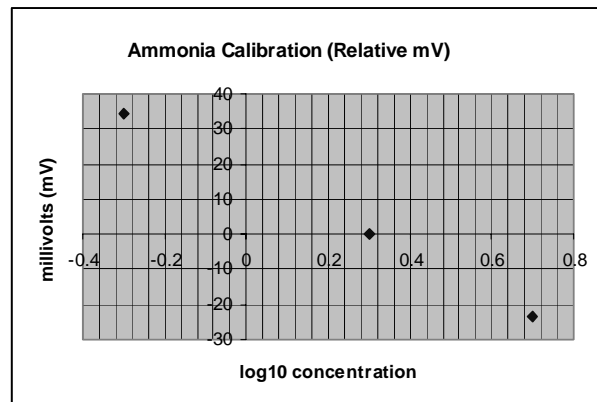
Step 3. Take the inverse log of the result in Step 2: $10^{-0.75595} = \mathbf{0.175407 \text{ mg/L}}$

This is the same thing as 10 raised to the power of the result from Step 2

Sample Calibration and LOD Data from Tree City WWTP: Calibration Data

Concentration (ppm)	Millivolts
0	205.9
0.5	127.1
2.0	92.8
5.0	69.6

Slope= -57.45726 *should be -54 to -60*
Intercept = 109.887
Correlation coefficient= -0.99998



Concentration = $\text{antilog} \{ \text{mV} \times \text{Slope} + \text{Intercept} \}$
or = $10^{(\text{mV} \times \text{slope} + \text{intercept})}$

Note: Because the LOD is significantly lower than the lowest calibration standard, the concentration of the lowest calibration standard should be reduced.

LOD Determination Data

Ammonia

Spike level= 0.25 mg/L

Rep. 1 0.25
Rep. 2 0.28
Rep. 3 0.25
Rep. 4 0.26
Rep. 5 0.27
Rep. 6 0.23
Rep. 7 0.26

# replicates	t-value
7	3.143
8	2.998
9	2.896
10	2.821

mean 0.257143

st dev. 0.016036

t-value 3.143 = from table based on # replicates

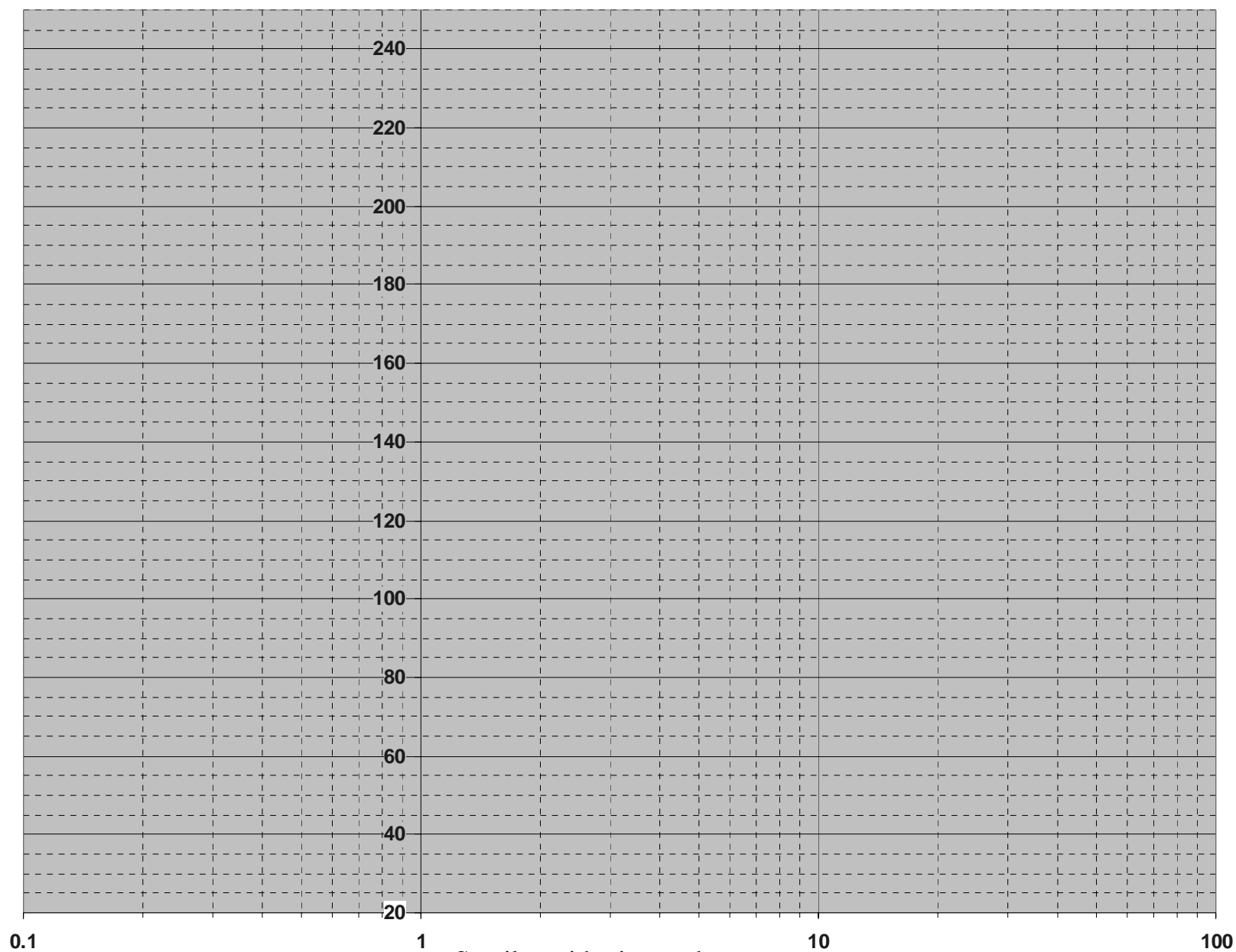
MDL 0.0504 = t-value x std deviation

LOD 0.0504 = roughly = to MDL

LOQ 0.168 = 10/3 x the LOD

The 5-point check

- 1 Is the MDL no lower than 10% of the spike level? **yes**
- 2 Is the spike level greater than the calculated MDL? **yes**
- 3 Is the MDL below any relevant permit limit? **N/A**
(if there is one) Permit limit=
- 4 Is the signal-to-noise ratio (S/N) between 2.5 and 10? **CHECK**
S/N = Mean/std dev. S/N= 16.04
S/N is fairly high which suggests a need to spike at a lower concentration
- 5 Is mean recovery within reasonably expected limits? **yes**
Mean recovery= mean/spike level x 100 102.86%



Semilogarithmic graph paper
make copies for use in the laboratory

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Determination of Total Phosphorus

Persulfate digestion followed by Ascorbic acid Method

Reference: Standard Methods, 18th edition, Procedure 4500-P B(5) & 4500-P E

Apparatus:

1. Hot plate (*An autoclave may be used in place of a hot plate.*)
2. Bausch & Lomb Spectronic 100 spectrophotometer or equivalent
3. Cuvettes. *If multiple cuvettes are used, they should be a matched set.*

Note: Use only glassware designated for phosphorus analyses. Wash glassware with a phosphate-free detergent and 1:1 hydrochloric acid after each use.

Reagents:

1. Phenolphthalein indicator
2. **Sulfuric acid solution (30%)**: Slowly add 300 ml conc. H_2SO_4 to approximately 600 ml distilled water. Cool and dilute to 1 L.
3. Ammonium persulfate: $(\text{NH}_4)_2\text{O}_8$, solid
4. Sodium hydroxide, 1N
5. **Stock Phosphate solution, 50 mg/L**: Dissolve 219.5 mg (0.2195 g) anhydrous KH_2PO_4 in reagent water. Dilute to 1 L. **1 ml = 50.0 ug (0.050 mg) $\text{PO}_4^{3-}\text{-P}$ [as phosphorous].**

NOTE: It is recommended that KH_2PO_4 be purchased from two different suppliers. The material from one source should be used to prepare calibration stock standards, while the other source is used to prepare stock standards used to prepare spike samples. If the same solution that is used to prepare calibration standards is also used to prepare spiked samples, errors made in the preparation of the stock standard cannot be easily identified. (Commercially prepared standard solutions may be purchased with certified concentrations).

6. **Standard Phosphate solution, 2.5 mg/L** : Pipette 25.0 ml of the stock solution into a 500 ml volumetric flask. Bring to volume with reagent water. **1.00 ml = 2.5 ug (0.0025 mg) $\text{PO}_4^{3-}\text{-P}$ [as phosphorous].**
7. **Potassium antimonyl tartrate reagent**: Dissolve 1.3715 g $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O} \cdot \frac{1}{2}\text{H}_2\text{O}$ in 400 ml reagent water in a 500 ml volumetric flask. Dilute to volume. Store in a glass-stoppered bottle.
8. **Ammonium molybdate reagent**: Dissolve 20 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 500 ml reagent water. Store in a glass-stoppered bottle.
9. **Ascorbic acid, 0.01M**: Dissolve 1.76 g ascorbic acid in 100 ml reagent water. **Store at 4°C. Discard after one week.**
10. **Sulfuric acid, 5N**: Dilute 70 ml conc. H_2SO_4 to 500 ml with reagent water.
11. **Combined reagent (Color Reagent)**: For 100 ml combined reagent mix in this order 50 ml 5N sulfuric acid, 5 ml potassium antimonyl tartrate reagent, 15 ml ammonium molybdate reagent, and 30 ml ascorbic acid. Let all reagents reach room temperature before combining. Mix the solutions well after each reagent is added. If the solution turns cloudy after mixing, let stand until it is clear. **This reagent must be used within four hours of preparation**
12. **Color Blank Reagent**: *Prepare only if necessary—see section 4.* For 100 ml combined reagent mix in this order: 35 ml reagent water; 50 ml 5N sulfuric acid; and 15 ml ammonium molybdate reagent. Let all reagents reach room temperature before combining. Mix the solutions well after each reagent is added. If the solution turns cloudy after mixing, let stand until it is clear. **This reagent must be used within four hours of preparation**

Calibration:

1. Preparation of Standard Curve *Make a new standard curve every three months or when reagents are replaced or whenever a check standard is not within 10 % of true value.* If a full set of calibration standards is not prepared on each day samples are digested, then at least one known standard, equal to the midpoint calibration standard and prepared from the stocks used to prepare calibration standards, must be prepared. If the result obtained for this known standard is not within 10% of the "true", or prepared, concentration, then a full calibration is required and samples must be re-digested.
 - a. Prepare at least three standards plus a blank (*Note: Standard Methods procedure calls for six standards*) at concentrations which bracket the concentration of the sample measured. *This analysis has been demonstrated to be substantially non-linear beyond 1.0 mg/L. Consequently, although some newer spectrophotometers are able to extend the linear range, you should limit your calibration to an upper end of 1.0 mg/L.*
 - b. Digest and test calibration standards in the same manner as the samples. *Since the EPA is no longer requiring that calibration standards be digested, undigested standards are allowed provided that a mid-point known standard is prepared and digested with each set of samples processed. If the recovery of this digested standard is not within 90% to 110%, there is indication that the digestion process significantly impacts results, and calibration standards should be digested as well.* Plot absorbance vs mg/L phosphate in standard to give a straight line.

NOTE: The procedure in Standard Methods suggests that this line should be drawn through the origin (0,0).

2. Digestion:

The persulfate digestion is the only digestion technique approved under NR 219, with the exception of the block digestion technique used by Technicon® or Lachat® automated analyzers. An option is given to use either a hotplate or an autoclave for the digestion.

- Hotplate Digestion
Boil all treated samples, standards, and blanks for 30-40 minutes or until a final volume of 10 mL is reached (whichever comes first—but keep in mind that some organophosphorus compounds can take as long as 1.5-2 hours to break down to orthophosphate).
- Autoclave Digestion
Autoclave for 30 minutes in an autoclave or pressure cooker. Set the conditions for 15-20 psi. (98-137 kPa) Samples are not boiled dry. **Note:** *there is little or no volume reduction with this technique. Keep this in mind when preparing matrix spikes.*

With both techniques, samples, standards, and blanks are allowed to cool following the digestion. A drop of phenolphthalein indicator is added and the samples neutralized by adding 1N NaOH dropwise until a faint pink color is achieved (*this will be pH 7.0 ± 0.2*). Dilute to 100 mL, but don't filter.

- a. Pipet a suitable portion of thoroughly mixed sample into a 250 ml Erlenmeyer flask.

NOTE: Use 2.0 ml of raw effluent, 10.0 ml of final effluent

- b. Dilute to 50 ml (if less than 50 mL is used).
- c. Pipette 50 mL of a standard into a 250 mL Erlenmeyer. It is best to vary the concentration of the standard as a check on different points on the calibration curve.
- d. Prepare a blank using 50 ml reagent water.
- e. Add one drop phenolphthalein solution to each flask. If a red color develops, use a dropper to add sulfuric acid solution (30%), one drop at a time, until the red color is gone.
- f. Add one more mL of sulfuric acid solution (30%) to the flask.

- g. Add one glass scoop (calibrated to = 0.4 g) ammonium persulfate. *You may wish to pre-weigh out several aliquots of 0.4 g on disposable "weigh boats".*
- h. Put the flasks on a hot plate. *Be careful when transporting beakers! To avoid contaminating samples with phosphorus, you should either wear laboratory gloves; in any case, do not contact the inside of the beaker/flask.* Boil slowly for 30 to 40 minutes or until the volume in the flask is reduced to approximately 10 mL. (**Do not allow to go to dryness.** If samples boil dry, you must discard that sample and start over.)
- i. Remove the flasks from the hot plate. Cool to room temperature.
- j. Add distilled water to the flask until the volume is approximately 30 mL.
- k. Add one drop phenolphthalein solution.
- l. Add sodium hydroxide solution with a dropper one drop at a time until a faint (light) pink color appears. Do not add excess NaOH.
- m. Transfer the sample into a 100 mL volumetric flask; dilute to volume with reagent water.

3. Color Development

- a. Pipet 50 mL digested sample into an Erlenmeyer flask. If you know or suspect the sample to contain appreciable concentration of phosphorus, use an aliquot of sample that has been diluted to 50 mL with reagent water (e.g., if your expected sample concentration is 3 mg/L, you may wish to dilute 10 mL of digested sample to 50 mL with reagent water). Be sure to account for any such dilution when calculating sample results.
***Note:** By "coloring", at most, 50 mL of the digested sample, you will have sufficient volume remaining to prepare a dilution if the sample response exceeds your calibration range.*
- b. Add 8.0 mL combined reagent to the 50 mL sample. Mix thoroughly.
- c. Allow the color to develop for **at least 10 minutes but not longer than 30 minutes.**
- e. Set the absorbance to zero using a digested reagent water blank. If any appreciable blue color is observed in this "calibration blank", it should be noted, and corrective action should be initiated to identify the source of contamination.

NOTE: This procedure assumes that calibration standards are digested. If they are not digested, zero the spectrophotometer each day of analysis with an undigested (like the standards) reagent water blank to which color reagent has been added

- f. Wipe the outside of each cuvette with a Kimwipe or soft tissue before inserting into the spectrophotometer. *Use the same cuvette for all blanks, standards, and samples. Different cuvettes may be differentially dirty or scratched leading to differences in baseline absorbance. This leads to bias in the analytical data, or may affect the ability to meet quality control limits. If multiple cuvettes are used, they should be a matched set.*
 - g. Rinse the cuvette between samples using the next sample to be tested.
 - h. The spectrophotometer should be set at 880 nm.
 - i. Read and RECORD the absorbance.
4. Calculate phosphorus concentration in sample as follows:

$$\text{mg/L total P} = \frac{\text{mg P (from curve)}}{\text{L}} \times \frac{\text{V mL}}{1000 \text{ mL}} \times \frac{1 \text{ L}}{1000 \text{ mL}} \times \frac{\text{FV mL}}{\text{CV mL}} \times \frac{1}{\text{SV mL}} \times \frac{1000 \text{ mL}}{\text{L}}$$

where

V = volume (mL) of sample + reagent water that was colored [typically 50]

FV = final volume (mL) after digestion [typically 100]

CV = volume (mL) of sample that was colored [typically 50]

if you used 10 mL diluted to 50 with reagent water, A= 10

SV = original volume of sample that was digested [typically 50]

By canceling out units, this formula can be simplified to:

$$\text{mg/L total P} = \frac{\text{mg P (from curve)}}{\text{L}} \times \frac{\text{V} \times \text{FV}}{\text{CV} \times \text{SV}}$$

NOTE: If you digest 50 mL of sample, dilute to a final volume of 100 mL, take 50 mLs of the digested sample and color it, the equation simplifies to:

$$\text{mg/L total P} = \frac{\text{mg P (from curve)}}{\text{L}} \times 2$$

Example: The following illustrates the calculation for an influent sample which measured 0.5 mg/L based on comparison of the sample absorbance to the calibration curve. A 50 mL sample (SV) was digested and diluted to a final volume of 100 mL (FV). A 10 mL (CV) aliquot of the digested sample was diluted to 50 mL (V) prior to the addition of color reagent.

$$\text{mg/L total P} = \frac{0.5 \text{ mg}}{\text{L}} \times \frac{50 \text{ mL}}{1000 \text{ mL}} \times \frac{1 \text{ L}}{10 \text{ mL}} \times \frac{100 \text{ mL}}{50 \text{ mL}} \times \frac{1}{50 \text{ mL}} \times \frac{1000 \text{ mL}}{\text{L}} = 5.0 \text{ mg/L}$$

5. The use of "color" blanks (if the sample has appreciable color following digestion)

Some plants analyze samples that seasonally develop color due to algae or other things. This type of color in a sample will register background absorbance on the phosphorus analysis and therefore must be subtracted from the true sample signal. This requires determining the absorbance of a "color blank".

A second aliquot of the digested sample should be colored with this solution and the **absorbance recorded should be subtracted from the absorbance obtained from the aliquot of digested sample** to which true color reagent was added.

Follow the procedure in steps 3 a and 3 b. It is important to use precisely the same amount of digested sample for the "color blank" as used for the actual sample analysis.

Sample Calibration and LOD Data from Tree City WWTP:

Calibration Data

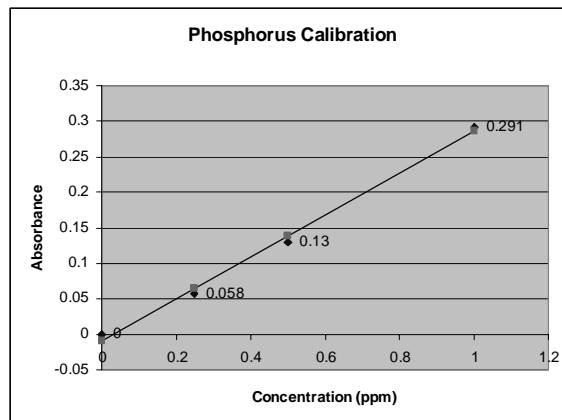
Concentration (ppm)	Absorbance
0	0
0.25	0.058
0.5	0.130
1.0	0.291

Slope= 3.38212347

Intercept = 0.03249071

Correlation coefficient= 0.99765256

$$\text{Concentration} = \frac{\text{Absorbance} - \text{Intercept}}{\text{Slope}}$$



LOD Determination

Total Phosphorus

Spike level= **0.1 mg/L**

Rep. 1 0.11
Rep. 2 0.12
Rep. 3 0.12
Rep. 4 0.12
Rep. 5 0.12
Rep. 6 0.11
Rep. 7 0.12

# replicates	t-value
7	3.143
8	2.998
9	2.896
10	2.821

mean 0.11714

st dev. **0.00488**

t-value **3.143** = from table based on # replicates

MDL **0.01534** = t-value x std deviation

LOD **0.01534** = roughly = to MDL

LOQ **0.05112** = 10/3 x the LOD

The 5-point check

- 1 Is the MDL no lower than 10% of the spike level? **yes**
- 2 Is the spike level greater than the calculated MDL? **yes**
- 3 Is the MDL below any relevant permit limit? **N/A**
(if there is one) Permit limit=
- 4 Is the signal-to-noise ratio (S/N) between 2.5 and 10? **CHECK**
S/N = Mean/std dev. S/N= 24.01
S/N is fairly high which suggests a need to spike at a lower concentration
- 5 Is mean recovery within reasonably expected limits? **yes**
Mean recovery= mean/spike level x 100 117.14%

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Determination of Chlorine Residual

DPD Colorimetric Method

Reference: Standard Methods, Procedure 4500-Cl G

A variety of approved methods are available to measure chlorine residual besides the procedures listed here. These methods include (Method numbers refer to 18th edition of Standard Methods):

1. The Iodometric Titration Method II (method 4500-Cl C) using amperometric endpoint detection (4500-Cl D). (The starch-iodine endpoint does not yield adequate detection limits required to consistently meet permit limits of 0.037 to 0.1 mg/L.);
2. DPD titrimetric or colorimetric methods (4500-Cl F and 4500-Cl G, respectively);
3. The specific ion electrode method produced by Orion Research, Inc. (not described in Standard Methods).

Kits are commercially available for the DPD method. If the laboratory uses one of these kits, the following requirements should be met:

- A spectrophotometer or filter photometer, equipped for use at 515 nm and providing a light path of at least 1 cm is needed to "read" the color.
- Total residual chlorine must be measured.
- Correction must be made for color and turbidity.
- The kit must have the ability to check for interference due to oxidized manganese (usually done by performing the test with and without adding sodium arsenite reagent).

Sample Collection:

1. Before collection combine the first three chemicals as specified in the procedure below to reduce chlorine loss.
2. Collect sample immediately prior to returning to the lab.
3. Sample must be a grab.
4. Sample should not be mixed or unnecessarily agitated after collection.

Apparatus:

1. Bausch & Lomb Spectronic 100 spectrophotometer or equivalent
2. Buret, capable of reading accurately to 0.02 mL.

Reagents:

1. **Phosphate buffer solution:** Dissolve 24 g anhydrous Na_2HPO_4 and 46 g anhydrous KH_2PO_4 in distilled water. Combine with 100 mL of distilled water in which 800 g *disodium ethylenediamine tetracetate dihydrate (EDTA)* have been dissolved. Dilute to 1 L with distilled water and add 0.02 g *mercuric chloride (HgCl_2)*. **CAUTION: HgCl_2 is toxic - take care to avoid ingestion.**
2. **N,N-Diethyl-p-phenylenediamine (DPD) indicator solution:** Dissolve 1 g DPD oxalate in chlorine-free distilled water containing 8 mL 1 + 3 H_2SO_4 and 200 g disodium EDTA. Dilute to 1 L, and store in a brown glass bottle in the dark. Periodically check solution blank for absorbance and discard when absorbance at 515 nm exceeds 0.002/cm. **Discard when colored! DPD oxalate is toxic - take care to avoid ingestion.**

NOTE: Use Eastman chemical 7102 or equivalent. Alternatively, use :

- 1.5 g DPD sulfate pentahydrate ($\bullet 5\text{H}_2\text{O}$), or
- 1.1 g anhydrous DPD sulfate

3. **Standard Ferrous ammonium sulfate titrant (FAS):** Dissolve 0.1106 g $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in distilled water containing 1 mL 1 + 3 H_2SO_4 and dilute to 1 L with freshly boiled and cooled distilled water. This standard may be used for 1 month. The FAS titrant is equivalent to 0.01 mg Cl (as Cl_2) per 1.00 mL.
4. **Potassium Iodide.** KI, crystals.
5. **Stock Potassium permanganate solution:** Place 0.891 g KMnO_4 in a volumetric flask and dilute to 1 L.
6. **Standard Potassium permanganate solution:** Dilute 1.0 mL of stock KMnO_4 solution to 100 mL with distilled water in a volumetric flask. 1 mL = 0.002 mg Cl (as Cl_2). 1 mL of this solution diluted to 100 mL with distilled water yields a chlorine equivalent of 0.1 mg/L.
7. **Sodium arsenite solution:** Dissolve 0.5 g NaAsO_2 in distilled water and dilute to 100 mL.

Calibration:

1. Prepare a series of permanganate standards having an equivalent chlorine range from 0.02 to 0.5 or 1.0 mg/L using the following table. Calibration should be performed any time reagents are changed.

Volume (mLs) Standard KMnO ₄	Final Volume (mLs)	Chlorine Equivalent (mg/L)
0.20	100	0.02
0.50	100	0.05
1.0	100	0.1
5.0	100	0.5
10.0	100	1.0

2. Place 5 mL phosphate buffer in each flask (250 mL). Add 5 mL DPD reagent.
3. Add 100 mL of the lowest permanganate standard.
4. Fill a cuvette from the flask and record the absorbance at 515 nm. Return the contents of the cuvette to the flask.
5. Titrate the contents of the flask with ferrous ammonium sulfate (FAS) until the red color is gone. Record the volume of FAS used.
6. Repeat steps 4 through 6 for each of the other calibration standards in order of increasing concentration. **For each standard, the volume of FAS titrant used (mLs) is equivalent to the chlorine concentration mg/L.**

Sample Procedure:

If the sample is suspected to contain oxidized manganese, add 0.5 mL phosphate buffer, 0.05 mL (about 1 drop, using a disposable dropper) sodium arsenite solution, and 10 mL sample. Mix these contents before adding the other reagents. Read the absorbance at 515 nm and subtract this from the sample absorbance obtained without the addition of the sodium arsenite.

1. Place 0.5 mL phosphate buffer in a 250 mL flask.
2. Add 0.5 mL DPD reagent.
3. Add approximately 0.1 g potassium iodide (KI) crystals.
4. Add 10 mL of sample. Let stand for 2 minutes.
5. Fill a cuvette from the flask and record the absorbance at 515 nm.

Calculations:

1. Plot the absorbance of KMnO₄ standards on the vertical axis vs. the titrated concentration (mg/L Cl₂) on the horizontal axis. Use this plot to determine chlorine residual concentration from sample absorbance. Alternatively, a regression can be calculated. *Refer to the phosphorus procedure for details.*

Determination of Chlorine Residual

Electrode (Iodometric) Method

Reference: Standard Methods, Procedure 4500-Cl I

Apparatus:

1. **Electrodes:** Either a combination electrode consisting of a platinum electrode and an iodide ion-selective electrode, or two individual electrodes.
2. **pH/millivolt ion meter:** Use an expanded scale pH/millivolt meter with 0.1 mV readability or a direct-reading selective ion meter.

Reagents:

1. **pH 4 buffer solution:** Dissolve 146 g anhydrous sodium acetate $\text{NaC}_2\text{H}_3\text{O}_2$ or 243 g $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ in 400 mL distilled water. Add 480 g concentrated acetic acid, dilute to 1 L with chlorine-demand-free water.
2. **Chlorine-demand-free water:** Add sufficient chlorine to distilled or de-ionized water to give 5 mg/L free chlorine. After standing 2 days, this solution should contain at least 2 mg/L free chlorine; if not, discard and obtain better quality water. Remove remaining free chlorine by placing container in direct sunlight or irradiating with an ultraviolet (UV) lamp. Keep checking residual chlorine; do not use until all traces of chlorine have been removed. *Standard Methods provides discussion on how to store chlorine-demand-free water for extended periods.*
3. **Potassium Iodide (KI) solution.** Dissolve 42 g potassium iodide, KI, and 0.2 g sodium carbonate, Na_2CO_3 , in 500 mL chlorine-demand-free, distilled water. Store in a dark bottle.
4. **Standard Potassium iodate solution (0.002 81 N):** Dissolve 0.1002 g potassium iodate (KIO_3) in chlorine-demand-free, distilled water, and dilute to 1000 mL. Each 1.0 mL, when diluted to 100 mL, produces a solution equivalent to 1.0 mg/L as Cl_2 .

Calibration:

1. Prepare a series of iodate standards using the following table. Calibration should be performed any time reagents are changed.

Volume (mLs) Standard KIO_3	Final Volume (mLs)	Chlorine Equivalent (mg/L)
0	100	0.0
0.1	100	0.1
0.5	100	0.5
1.0	100	1.0
5.0	100	5.0

2. Pipet 1 mL acetate buffer and 1 mL KI solution into each flask (250 mL). Stopper, mix, and let stand 2 minutes before diluting to volume (100 mL) with chlorine-demand-free, distilled water.
3. In order of increasing concentration—starting with the lowest concentration standard—pour the flask into a 150 mL beaker. Stir gently, without creating turbulence, and immerse electrodes.
4. Wait for the potential to stabilize and record the potential in millivolts (mV).
5. Repeat this procedure for the other standards and then the reagent blank.
6. Prepare a calibration plot or regression as is done for ammonia (*refer to the Ammonia procedure*).

Sample Procedure:

1. Select a volume of sample not to exceed 100 mL which will not exceed the calibration range, and pipet into a 100 mL volumetric flask.
2. Pipet 1 mL of acetate buffer and 1 mL of KI solution into the flask. Stopper, mix, and let stand 2 minutes.
3. Adjust sample pH to between 4 and 5 if necessary, using acetic acid. Dilute to the mark with sample (if using 100 mL of sample) or with chlorine-demand-free water if using an aliquot of sample less than 100 mL. Stopper, mix, and let stand 2 minutes.
4. Pour the flask into a 150 mL beaker. Stir gently, without creating turbulence, and immerse electrodes.
5. Wait for the potential to stabilize and record the potential in millivolts (mV). If the millivolt reading exceeds that of the highest concentration calibration standard, repeat analysis with an appropriately smaller aliquot of sample.

Calculations:

1. Determine chlorine concentration in mg/L (A) from either the calibration plot, or a regression equation. Total residual chlorine is then calculated as:

$$\text{Total residual chlorine} = \frac{A}{V} \times 100$$

Where V = sample volume used (in mL). If total residual chlorine is below 0.2 mg/L, subtract apparent chlorine in reagent blank to obtain the true total chlorine residual.

Determination of Fecal Coliform

Membrane Filter Fecal Coliform Test (MFFCC)

Reference: Standard Methods, 18th edition, Procedure 9222 D

Pre-Sampling Preparation:

It is best to run the residual chlorine test immediately before the fecal test to better anticipate the number of coliform and correlate the 2 parameters.

1. Prepare phosphate or peptone buffer water. Preferably dispense about 100 ml of buffer into milk dilution bottles and cap loosely. Sterilize.
2. Select a sample container of either hard glass (pyrex) or nontoxic plastic (polypropylene), preferably with a wide mouth and at least 100 ml. in size. Deliver 0.1 ml. of 10% sodium thiosulfate solution to sample container for each 100 ml. to be collected. This will neutralize any chlorine present. Cap container loosely and sterilize.
3. Sterilize the funnel unit of the filtering assembly.
4. Sterilize all pipets and graduated cylinders to be used.

Sampling:

1. Sample must be collected directly into the sterile sample bottle. The sample bottle should not be filled to the top so it can be shaken and mixed well during the test. Also, if overflow occurs the sodium thiosulfate may be lost and chlorine will not be dissipated.
2. The test should be set up within 6 hours of sample collection. If samples must be sent out for testing they must be collected as described above and must be iced and/or refrigerated during transit to the lab. The maximum time between sample collection and testing is 24 hours.

Testing:

1. Disinfect work area with alcohol solution. *The alcohol may be isopropyl or rubbing alcohol purchased at a drug store.*
2. Label each sterile petri plate with sample volume to be filtered.
3. Choose sample volumes based on expected number of fecal coliform. Volumes should cover a range which results in at least one plate having 20-60 colonies.

NOTE: To obtain proper counts on culture dishes, several dilutions should generally be used.

- Standard Methods recommends using 10, 1, and 0.1 mL for secondary effluent
- Standard Methods recommends using 0.1, 0.01, and 0.001 mL for raw municipal sewage.

4. Handle sterile pads and filters with a forceps dipped in alcohol and flamed off in a bunsen burner or alcohol lamp. Only the outer 1/8 inch of the filter should be touched with the forceps.
5. Place a sterile absorbent pad in each plate. Tap the contents of an MFFCC ampoule onto the pad and pour off any excess liquid. *Pre-mixed ampoules and sterile pads are used for media in culture dishes.*
6. Place the sterile filter on the funnel base. Carefully put the funnel in place.
7. Pour 20-30 ml of the buffer water onto the filter.
8. Shake the sample very vigorously before withdrawing each volume. Add the smallest volume to be filtered to the buffer water in the funnel. Apply a suction of not more than 20 psi. In 2 successive additions of buffer water (20-30 ml) rinse down the sides of the funnel. Suction until dry.

9. Carefully remove the funnel and lift membrane into petri dish with sterile forceps. The membrane filter should be in contact with the pad with no air bubbles underneath. If air bubbles occur, lift the membrane on one side and lower slowly. Do not touch the filtered area with the forceps.
10. Repeat with next largest sample volume.
11. To incubate, place petri dishes in a water proof plastic bag ("Ziploc" or "Whirl-Pac") and seal. Invert the bag and secure under water in a water bath at $44.5 \pm 0.2^{\circ}\text{C}$. The thermometer in the bath must read to a tenth of a degree. *A solid block desk-top incubator is often used.*
12. Incubate the plates for 24 " 2 hrs. Remove the plates from the bag. Select those plate(s) that contain 20-60 blue colonies. Average those plates in this range or, if only one plate has 20-60 use that. If no plates fall in this range count those which are nearest to 20-60.

Calculation:

$$\text{Fecal coliform/100 ml} = \frac{100 \times \# \text{ colonies counted}}{\text{number of ml filtered}}$$

Determination of pH

Electrometric Technique

Reference: Standard Methods, 18 edition, Procedure 4500-H⁺ B
Electrode manufacturer's manual

Apparatus:

1. pH Meter, Corning 7 (or equivalent)
2. Combination electrode, Orion model 91-05 (or equivalent)
3. Stirring apparatus (optional)

Reagents:

1. Buffers, pH values 4, 7, and 10. Purchased commercially.

Sample Collection:

1. Samples are collected in clean plastic bottles and brought immediately to the lab for pH measurement.

Calibration:

These calibration instructions apply only to this particular pH meter model. You should consult the manufacturer's instructions for directions on how to calibrate your pH meter. In all cases, the meter should be calibrated with two buffers which bracket the pH measured, and then verified with a third buffer which falls between the two used for calibration.

NOTE: If only occasional measurements are made, calibrate before each measurement.

1. Switch the Function knob to Standby and set the Temperature Control to the temperature of the buffer. NOTE: The buffers and samples must all be at the same temperature.

NOTE: *Not all manufacturers suggest stirring samples, but it can improve electrode response time and sample homogeneity. If samples are stirred, standards should be stirred in the same fashion. Some magnetic stirrers generate enough heat to increase the temperature of the sample. To avoid this, place a piece of cardboard between the stirrer and the beaker.*

2. Immerse the electrode into the **pH 4 buffer** and switch the Function knob to the pH mode. With the Normal/Expand knob in the Normal position, the meter should be adjusted to read **4.0**, on the normal scale, using the Calibrate knob.
3. Switch the Function knob to Standby, remove the electrode from the buffer. Rinse with distilled water.
4. Immerse the electrode into the **second buffer** solution (suggested **pH 10** so that the buffers bracket the pH measured).
5. Switch the Function knob to the pH mode. With the Normal/Expand in the Normal position, adjust the meter to read **pH 10.0** on the normal scale, using the Temperature Control.

NOTE: If the second buffer requires more than a 2°C adjustment of the Temperature Control, re-check the temperature of the buffer solutions, or replace the buffers with fresh solution, and repeat the calibration.

6. Switch the Function switch to Standby, remove the electrode from the solution and rinse with distilled water. Store the electrode in 4 M KCL. *The electrode storing solution varies with the type of electrode used. To prevent faster deterioration of the electrode (and more costs to the lab), store the electrode in the storing solution recommended by the manufacturer.*
7. Using a third buffer which falls between the two used for calibration(e.g.; **pH 7.0**), verify the accuracy of the calibration. Immerse the electrode into the **verification buffer** solution (**pH 7**, a value that falls within the calibration range).

8. Switch the Function knob to the pH mode. Wait for the reading to stabilize (usually no more than two to three minutes). Read and record the pH value to the nearest 0.1 unit.
9. If the measured pH of the verification buffer falls within ± 0.1 pH units of the expected value (i.e., 6.90 to 7.10), then the calibration is acceptable, and you can move forward with sample analysis.

NOTE: *If your pH meter does not allow calibration with pH buffers that are more than 3 pH units apart, you can calibrate with the 4 and 7 and then check the calibration using either the 4, 7, or some other buffer between the two. Alternatively, for samples with pH values between 7 and 10, you can calibrate with the 7 and 10 and then check the calibration using either the 7, 10, or some other buffer between the two.*

Procedure:

1. Standardize the electrode as described above.
2. Place the sample in a clean glass beaker. Use a sufficient volume to cover the sensing elements of the electrode.
3. Immerse the electrode in the sample. Wait for the reading to stabilize (usually no more than two to three minutes.) *Samples and pH buffer standards are not stirred during measurement. The stirring action causes carbon dioxide to be absorbed into the sample which can affect pH readings in samples which lack adequate buffering capacity(i.e., low alkalinities).*

NOTE: typical domestic wastewaters throughout most of the state cannot be characterized as having low alkalinities.

4. Read and record the pH value to the nearest 0.1 unit.
5. Rinse the electrode with distilled water. Store in 4 M KCl (*or as recommended by the manufacturer*).